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HYDROGEN-RATED SYSTEM FOR *IN VITRO* STUDIES AT PRESSURE: OPERATING PROCEDURES AND EMERGENCY PROCEDURES

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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A special apparatus was constructed to study the neurophysiologic effect of high pressure and the pharmacology of various gaseous agents using the isolated nerve terminal (synaptosome) tissue preparation. Design features were incorporated to permit investigations using H_2, among other gases. Detailed operating procedures and emergency procedures for the use of H_2 were established. These procedures are memorialized by this report. The theory of operation of the device and a pertinent overview of safety considerations are covered.

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INTRODUCTION

Synaptosomes are isolated nerve terminal endings which maintain many of the functional properties of the intact presynaptic neuron. As such, the synaptosome preparation is a useful model for study of presynaptic physiology. Synaptosomes have been used to investigate the effects of high pressure on nerve terminal Ca^{2+} uptake (1) and on K^{+}-depolarized (2-10), A23187 Ca^{2+} ionophore stimulated (11), and compression induced (12) neurotransmitter release.

The motivation for such studies would include as a goal the delineation of how pressure affects the central nervous system (CNS) to cause the high pressure nervous syndrome (HPNS). This is a debilitating condition that can impair the performance and threaten the safety of human divers at depths greater than 600 feet sea water (fsw), manifested by tremor, arousal deficits, and EEG changes (13,14). In animals exposed to even greater pressures, HPNS progresses to convulsions and death.

Many narcotic/anesthetic drugs ameliorate HPNS (15). Included among these are a number of gaseous agents. The potency of some of these gases is such that the pharmacologic activity becomes apparent only under hyperbaric conditions. N_2, the predominant constituent of air, is a noteworthy example. The addition of 5-10% N_2 to breathing mixture has been used as a countermeasure to HPNS in attaining record depths in human diving (16). The pharmacology of breathing gases is thus most relevant to the problem of high pressure effects on the CNS.

There has been a resurgence of interest in the utility of H_2 as a component of breathing mix for deep diving (17). Low density and other considerations confer practical advantages to the use H_2. Moreover, hyperbaric H_2 produces narcosis and ameliorates HPNS, intermediate in potency between He and N_2 (18,19). Human experiments confirm the utility of H_2 in alleviating HPNS (20, 21), but potentially adverse occurrences including apneic episodes have been observed (22). Consequently, the CNS action of hyperbaric H_2 becomes an object of intense scrutiny.

In order to investigate the pharmacology of various hyperbaric gases, Imbert and colleagues (23) designed a special apparatus to saturate liquids with defined gas mixtures at high pressure. These solutions are used as perfusion media for a tissue preparation, so that the tissue is exposed to dissolved gas at the partial pressure of each component in the gas mixture. The design includes safety features which permit utilization of H_2 as well as other gases.

The apparatus was constructed with a specific intent for utilization with the synaptosome preparation. Two separate gas-saturated physiologic electrolyte solutions are used for perfusing the synaptosomes. These solutions contain low K^+ and high K^+ respectively. Switching perfusion from the low K^+ to the high K^+ solution causes depolarization of the synaptosome membranes, inducing neurotransmitter release. Thereby, the time course of neurotransmitter release can be observed, subject to experimental manipulations of pressure and gas mixture.

While the principles of the device are straightforward, operation is complicated by stringent requirements for safety. This report memorializes the approved operating procedures (OP's) and emergency procedures (EP's) that have been worked out. The appendices contain the specific checklists for operation of the hyperbaric chamber and the equipment, both for H_2 and for less stringent non-H_2 dives. Photographs are included to define the nomenclature for the various panels, valves, and controls. Useful compression schedules are also appended.

THEORY OF OPERATION

Figure 1 is a schematic showing a liquid-containing vessel (hereafter referred to as the saturator vessel, saturator, or bubbler) inside a boundary which symbolizes a compression chamber (the hyperbaric chamber or chamber). The hyperbaric chamber is compressed with He through a tracking regulator and valve system (Compression) and decompressed through an exhaust valve (Decompression). He for compression is provided from laboratory supply lines and panel.

Pressure inside the saturator vessel is maintained differentially higher than the chamber pressure. Instrumentation to monitor this pressure difference is shown schematically as ΔP. A desired gas or mixture of gases (Gas) is bubbled through the liquid contained in the saturator. Flow rate of Gas is controlled by a tracking regulator (TR) and fine metering valve (FMV), and indicated on a ball flowmeter (BF). The bubbling action inside the

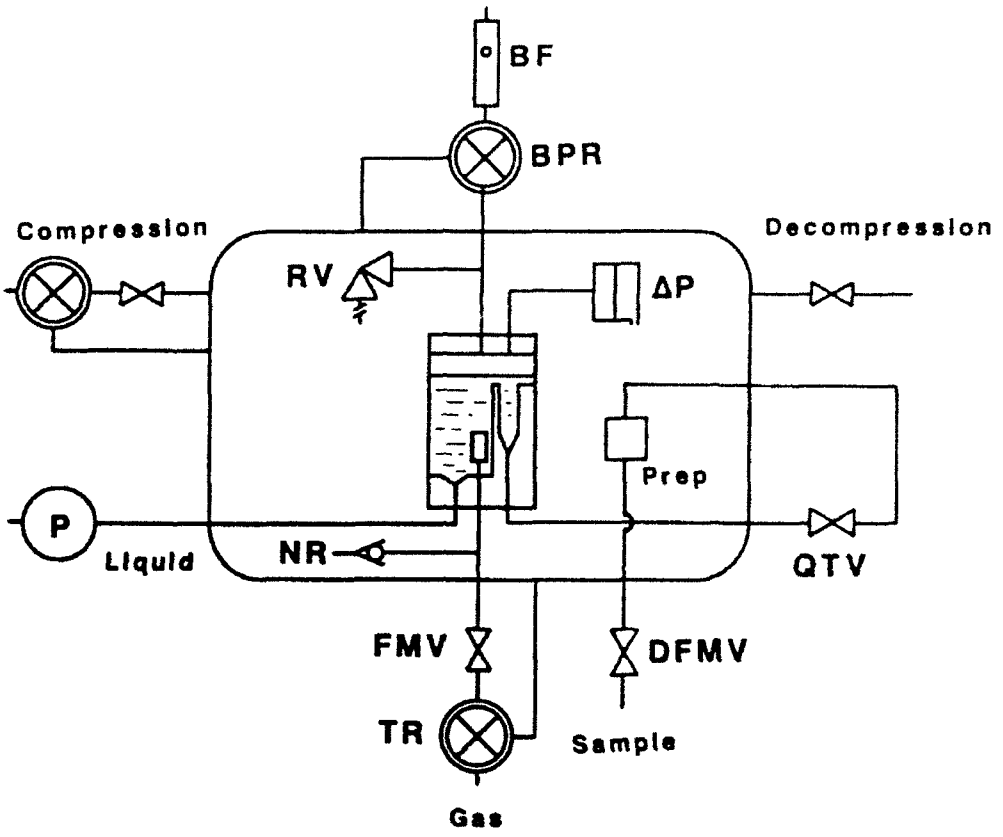


Figure 1. Pressure and flow control circuitry. The saturator is flushed with a gas mixture of selected composition via a tracking regulator (TR) and a fine metering valve (FMV). The gas bubbles through the liquid contained in the saturator. Gas flow is read on a ball flowmeter (BF)., while a back pressure regulator (BPR) controls a preset positive difference in saturator pressure (ΔP) read on a differential pressure gauge. Pressure provides the driving force for the liquid to be delivered to the tissue preparation. Liquid flow can be shut off by a quarter turn valve (QTV) to service Prep. A dual fine metering valve (DFMV) controls the perfusion flow rate. A high pressure pump (P) allows refilling of the saturator with liquid as required. A pressure relief valve (RV) and a non-return valve (NR) protect the saturator from excessive positive or negative ΔP. For picture clarity, stop valves are not represented. Reproduced from (23), with permission.

saturator vessel presents Gas to the liquid, so that the liquid ultimately becomes saturated with dissolved gases at the partial pressure of each component of the Gas mixture. Temperature of liquid in the bubbler is thermostatically controlled.

Liquid from the bubbler perfuses the tissue preparation (Prep). Thus, Prep is exposed to Gas, which saturates the perfusion liquid. Secretory products that are released by Prep are carried onward in the stream of perfusate. This stream is brought across the pressure boundary for collection by the experimenter (Sample) to be analyzed for such secretory products. Perfusion flow rate is controlled by a dual ganged fine metering valve (DFMV). Outflow from the bubbler can be shut off by a quarter turn valve (QTV) so that Prep can be serviced. In practice, duplicate saturator vessels are used to contain low K^+ and high K^+ solutions respectively. The choice of solution for perfusing Prep is selected by a valve. Liquid in the bubblers is replenished by a high pressure pump (P).

Gas is introduced to the saturator vessels and exhausted through lines that are isolated from the ambient chamber atmosphere. The gas volume within this internal system is small. Thus the amount of Gas inside the hyperbaric chamber -- as for example containing H_2 -- is minimized. An inerting line (not shown on the schematic) is incorporated in the exhaust system for addition of He to dilute the exhaust to a nonflammable level.

During compression or decompression of the chamber, the differential pressure ΔP inside the bubblers is maintained by a back pressure regulator (BPR). Note the reference line that

connects BPR to the chamber, by which ΔP is regulated with respect to the prevailing chamber pressure (similar reference lines connect to the tracking regulators). Fine adjustments to ΔP during compression are made by adjustment of BPR. Fine adjustments to Gas flow rate, as indicated on BF, are made by adjustment of FMV.

A relief valve (RV) and non-return valve (NR) are provided to prevent bursting or crushing of the bubblers in the event of loss of control of ΔP. These valves are set at 75 psi (RV) and 10 psi (NR). The exhaust of RV inside the chamber vents through water in a beaker that is visible to the chamber operator. The absence of bubbles at this orifice provides visual confirmation that gas inside the saturators is not leaking through to the ambient chamber atmosphere. Ability to maintain ΔP inside the saturators when Gas mixture supply and exhaust are shut off at the chamber stop valves (not depicted on the schematic) indicates the gas tight integrity of the internal system.

The supply of Gas is from a high pressure bottle ("K" bottle) connected to the system at a bottle manifold. Any of several Gas mixtures from different bottles may be selected at the manifold. One-way valves in the circuitry prevent inadvertent mixture from one source to another in the event of procedural error. The circuitry also provides means to flood the lines and the saturators with He from the laboratory supply to inert the system and remove any potentially explosive conditions.

SAFETY

H_2 is a colorless odorless gas that readily combines with O_2 to burn with an intensely hot invisible flame. The energy required to ignite H_2 is an order of magnitude lower than that required for ignition of gasoline (24). For example, a H_2 fire can be initiated by static electricity discharges or by minimal friction from mechanical sources (25). An H_2-O_2 mixture that is ignited in a confined space will likely cause an explosion.

As the smallest gas molecule, H_2 has a high propensity to leak from valves, joints, and fittings. Fittings therefore always pose a hazard as a potential originating point of a flame that can not be seen by an operator (25). Hook-up and disconnection of H_2 fittings and whips can be particularly hazardous. If the lines should contain H_2, an immediate source of ignition can arise from the mechanical friction of the moving coupling parts while H_2 is venting and mixing with air. Potential for burns or injury is great. Accordingly, flame proof gloves must be worn whenever H_2 fittings are handled. Tools should be made of low sparking copper-beryllium alloy.

Special care must be taken to ensure that connections do not leak (26). After connections are made, gas tight integrity of pressure fittings must be verified by using soap suds solution as a leak detector.

Adiabatic heating is another potential source of ignition when lines are charged with H_2. Lines and fittings must be purged with

inert gas to remove O_2 prior to introducing H_2. Prudence would also suggest that lines should be brought up to pressure in a slow deliberate fashion, rather than jammed up by sudden valve opening.

As a gas that is lighter than air, there is a potential for H_2 to collect in overhead pockets and crevices. This is preventable by adequate exhaust ventilation and by reasonable measures to circulate room air (26). For this purpose, a large exhaust hood and blower is installed in the laboratory above the pressure chamber to collect any escaped gas.

It is important that H_2 fires, should they occur, be brought under control by securing the H_2 at its source (26). The use of fire extinguishers on an H_2 flame can be dangerous because, although the fire may be put out, the continuing leak of H_2 can set the stage for an explosion.

Risk is also minimized by limiting the total quantity of compressed H_2 present in the laboratory. As a practical matter, no more than one "K" bottle of compressed H_2 mixture need be in the laboratory at any given time. Special hook-up hardware is installed to prevent improper connection, and only one H_2 connector is provided for the apparatus. Bottles should be maintained in outdoor open air storage when not in actual use in the laboratory. These practices conform to NFPA 50A, the relevant National Fire Protection Association standard (27).

Notwithstanding the propensity of H_2 to vigorously oxidize, atmospheres that contain less 4% H_2 and/or less than 4% O_2 will not

support H_2 combustion (28,29). This fact is exploited in order to use H_2 safely. For example, purging lines and inerting exhausts with He reduces the fractional concentration of O_2 or H_2 to safe levels. The exhaust line of the system is instrumented to indicate percent O_2 in the gas stream. By this means, a purging procedure or inerting procedure can be monitored to maintain O_2 at safe levels. OP's for the system are set forth in a way that causes the appropriate purges to occur prior to introduction of H_2 gas. Similarly, the room exhaust hood above the hyperbaric chamber is equipped with an alarmed H_2 detector to forewarn of leak conditions before reaching the lower flammability limit.

H_2 experiments typically employ hydrox mixtures in order to concurrently provide the required O_2 for tissue preparation viability. Of course, such mixtures must contain less than 4% O_2 to be nonexplosive. A typical hydrox mixture would be 2% O_2, balance H_2. At 10 atmospheres absolute pressure (ATA), this fractional concentration of O_2 provides a PO_2 of 0.2 ATA, roughly approximating the PO_2 of air. Thus, the aerobic requirements for tissue can be met, even when using a 2% hydrox mix, at a pressure of 10 ATA (roughly 300 fsw) or greater.

In similar vein, compression of the air-filled hyperbaric chamber from sea level to 300 fsw using He reduces the fractional concentration of the ambient chamber atmosphere to about 2%. Therefore, even in the event of catastrophic failure that permits H_2 to invade the chamber atmosphere, explosive conditions do not

arise (23).

A typical H_2 dive is initiated using a non-H_2 mix on the surface which has a high fractional O_2 concentration, e.g. 21%. A switch to the hydrox mixture is then effected from the gas bottle manifold as the chamber passes 300 fsw. Prior to the H_2 switch, the system must be purged by switching to a low O_2 gas mix. When the O_2 fraction indicated in the exhaust stream falls to a safe level, the switch to hydrox can be safely performed.

DISCUSSION

The system was designed to permit safe neurophysiologic experiments with H_2 and other gases at pressures up to 2000 fsw. The appended OP's and EP's were carefully constructed so that the logic of the design for safety purposes is captured in the execution of the sequential checklist. Notwithstanding, there is no substitute for an appreciation of the operating principles of the system and the various potential hazards. On the basis of such understanding, and together with the procedural checklists, the system has been successfully and safely utilized to conduct the kinds of experiments for which it was built.

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APPENDIX A

OPERATING PROCEDURES INVOLVING HYDROGEN GAS

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HYDROGEN-RATED SYSTEM FOR "IN VITRO" STUDIES AT PRESSURE

OPERATING PROCEDURES AND EMERGENCY PROCEDURES

Revised May 1992

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Synaptosome Chamber Dive Checklist (Rev May 92)
(filename \HJM\H2OP6.WP)

OPERATING PROCEDURES INVOLVING HYDROGEN GAS

Date: _____
Supervisor: _____
Chamber operator: _____

GENERAL SAFETY RULES:

GAS BOTTLES CONTAINING HYDROGEN WILL BE STORED IN THE OUTDOOR COMPOUND WHEN NOT IN USE. A SINGLE BOTTLE MAY BE BROUGHT INTO THE LABORATORY AND PLACED UNDER THE HOOD AFTER PROCEDURE 1 IS COMPLETED.

NO BOTTLE CONTAINING HYDROGEN SHALL BE VENTED OR OPENED PRECEDING WHIP HOOKUP.

AFTER CONNECTION TO THE WHIP, A GAS BOTTLE CONTAINING HYDROGEN WILL REMAIN CONNECTED UNTIL READY FOR REMOVAL FROM THE LABORATORY.

SYSTEM AND CHAMBER MUST BE PURGED WITH INERT GAS CONTAINING $\leq$ 2% O_2 BEFORE AND AFTER INTRODUCING HYDROGEN.

HYDROGEN MUST NOT BE INTRODUCED TO THE CHAMBER AT DEPTHS LESS THAN 330 FSW

A GAS BOTTLE CONTAINING HYDROGEN WILL BE DISCONNECTED FROM ITS WHIP USING PROCEDURE 8.

FOR ANY MALFUNCTION OR ABORT, FIRST STEP IS TO SECURE HYDROX BOTTLE VALVE. SEE EMERGENCY PROCEDURES.

FLAME PROOF GLOVES MUST BE WORN WHEN HANDLING HYDROX BOTTLE VALVE, CONNECTOR, OR WHIP.

BEWARE!!! HYDROGEN BURNS WITH INVISIBLE FLAME.

MONITOR ROOM H_2 LED DISPLAY AT ALL TIMES AFTER CONNECTING HYDROX BOTTLE, MUST BE < 25% LFL.

First dive of day is executed starting from Procedure 1.
Subsequent dives are executed starting from Procedure 4.
Shutdown after last dive is executed as Procedure 8.

For each procedure, chamber operator will initial each item as performed. Mark NR if step is determined not required by supervisor. For any discrepancies, do not proceed further until corrected or unless given permission by supervisor. Record discrepancies in chamber dive log and notify MA04.

1. PROCEDURE: Preliminary Checks and Valve Lineup

The purpose of this procedure is to ensure that high pressure is not inadvertently in the lines, that electrical connections for the thermostatic controllers are satisfactory, that gas detection and monitoring equipment is operating correctly, that the exhaust system is operating, and that all valves are in the correct initial position.

____ Notify gas farm 5-5910 to supply lab helium

Helium Supply Wall Panel
____ All valves closed (4 valves)
____ Regulator unloaded (blue knob CCW)
____ Upstream pressure 1,200 ± 100 psig (gauge 1)
____ Delivery pressure 0 (gauge 2)

Milton Roy Pump
____ Pump off
____ Pump flush valves closed (2 valves)
____ Pump select valves to drain (2 valves)

Mix Gas Bottles and Manifold
____ All gas bottles secured (hydrox bottle not present)
____ All gas bottle valves closed
____ All mix valves closed (2 green, 1 red)
____ Inerting valve closed
____ Bleed off valve closed
____ Gauge calibration port capped and valve open, turns freely
____ Manifold pressure gauge 0

Chamber Control Panel
____ Helium source valve closed (black)
____ Mix source valve closed (red)
____ All pressure gauges 0 (6 gauges)
____ Depth LED display illuminated, reads 0 ± 1 fsw
____ LED displays for exhaust O_2 and room H_2 are illuminated
____ Differential pressure LED display on, indicating 0 ± 0.2 psi
____ Mix flow vernier open, turns freely
____ Gauge calibration port capped and valve open, turns freely
____ Gauge bleed off valve closed (black)
____ Shunt valve closed (red)
____ Bleed off valves closed (red, 2 valves)
____ Compression valves closed (2 valves, X = coarse, black = fine)
____ Decompression valves closed (2 valves, X = coarse, black = fine)
____ Inerting valve closed (black)
____ Flow meter valve open, turns freely (black)

Top Chamber
____ Fast decompression line closed (2 quarter turn valves, both closed)

Left Side Chamber
____ Analysis valve closed
____ Depth gauge valve open
____ Reference valve open

_____ Decompression valve open
_____ Compression valve open
_____ Mix in valve open
_____ Mix out valve open
_____ Buffer 2 valve open
_____ Buffer 1 valve open

Synaptosome Perfusion Device (Inside Chamber)

_____ Electrical connectors installed (3 Cannon connectors interface to chamber)
_____ Temperature sensors (3 phono plugs) and heater elements (3 Jones plugs) connected
_____ Quick disconnect fittings installed (3 reds = mix in, 3 greens = mix out from top of water trap, mix out from each saturator, 2 violets = buffer 1 to R saturator, buffer 2 to L saturator)
_____ Temperature sensor installed in synaptosome heater block
_____ Bubbler outlet valves closed (2 valves, tagged yellow)
_____ Fill beaker at relief valve exhaust with water to a level above the exhaust outlet

Right Side Chamber

_____ Sample valve closed
_____ Ganged sample verniers open, turn freely (2 valves)

Electrical Equipment

_____ Turn on hydrogen hood exhaust fan, check that streamers are streamlining
_____ Sensidyne hydrogen monitor unit P (power) lamp is on and H,L,F (high,low,fail) lamps are off; mode switch is in "operate" position; LED display reads between 00 and 25 (not flashing, not -0) and corresponds to room H_2 LED display on chamber control panel
_____ Switch Teledyne oxygen monitor to HIGH range; meter indication should correspond to exhaust O_2 LED display on chamber control panel
_____ Turn on heater power supplies (3 each)
_____ Turn on temperature controllers (3 each) and check setting 37°
_____ Ensure that temperature controllers are cycling and that heating elements on perfusion device are not overheating

2. PROCEDURE: Drain and Refill Saturators;
Check Gas Tight Integrity of Saturators

The purpose of this procedure is to drain and refill liquid from the bubblers and to check that the bubblers will hold pressure.

2A. Drain saturators

Helium Supply Wall Panel

____ Provide helium supply. Open bypass valve, note pressure rise on delivery supply gauge

Chamber Control Panel

____ Open helium source valve, note pressure rise on helium pressure gauges (2 gauges, upper gauge shows supply pressure; lower gauge reflects loading of helium tracking regulator)

____ Open shunt valve, note pressure rise on mix pressure gauges (2 gauges, upper and lower gauge indications as above)

____ Establish pressure in saturators. Adjust back pressure regulator to about 10 psi (no more than 20 psi) on differential pressure LED display, check for flow in flowmeter as pressure builds up, visually check saturators for bubbling

___ Adjust mix flow vernier for flow rate 2 on flowmeter

Milton Rcy Pump

____ Drain the saturators. Open pump flush valves (2 valves), visually check for effluent in drain lines

____ When saturators are drained, close pump flush valves (2 valves)

Synaptosome Perfusion Device

____ Rigged for effluent to sampling line

____ Open bubbler outlet valves (2 valves, tagged yellow)

Right Side Chamber

____ Open sample valve, beaker for collecting effluent under sampling line

____ Bubbler select valve in either position 1 or 2

____ When saturator is drained, switch bubbler select valve to other saturator, allowing it to drain

____ When draining is completed, close sample valve, reposition bubbler select valve as desired

Left Side Chamber

____ System gas-tight integrity check. Simultaneously close mix in valve and mix out valve

_____ Note differential pressure on LED display

_____ Note differential pressure after 1 min, allowable pressure drop $\leq$ 1 psi

____ Open mix in and mix out valves simultaneously

Chamber Control Panel

____ Close shunt valve

2B. Fill Saturators

Milton Roy Pump

____ Prime Milton Roy pump. Turn on pump and prime with solutions of choice, adjusting pump vernier controls as required (2 knobs); pump select valves in drain position (2 valves); effluent flowing in drain lines

____ Refill saturators. After pump is primed, switch pump select valves to bubbler position (2 valves)

____ Visually monitor saturators to prevent overfilling

____ When saturators are filled, turn off Milton Roy pump

3. PROCEDURE: Connect Mix Gas Bottles and Check Pressures

The purpose of this procedure is to connect and determine the pressures of the mix gas bottles.

Bottles containing high O_2 (up to 25%) may be connected to whip #1.
Only bottles that contain ≤ 2% O_2 in inert gas may be connected to whip #2.
Bottles containing hydrox must be connected only to whip #3.

Bottle pressures (psig) must be 0.5 X depth of intended use (fsw) + 200 psi.

Bottles in standby condition should be closed at the bottle valve.

Chamber Control Panel
____ Mix source valve closed
Mix Gas Bottles and Manifold
____ Mix valves closed (3 valves), manifold pressure gauge 0

3A. Whip #1
____ Bottles containing high O_2 (up to 25%) may be connected to whip #1.
_____ Name/composition of mix
____ Open #1 bottle valve
____ Open mix #1 valve
_____ Record manifold pressure, must be 300 psig minimum
____ Close #1 bottle valve
____ "Bleeding down!" Open bleed off valve to bleed down, then close
___ Close mix #1 valve

3B. Whip #2
____ Only bottles that contain ≤ 2% O_2 in inert gas may be connected to whip #2.
_____ Name/composition of mix
____ Open #2 bottle valve
____ Open mix #2 valve
_____ Record manifold pressure, must be 1200 psig minimum
____ Close #2 bottle valve
____ "Bleeding down!" Open bleed off valve to bleed down, then close
____ Close mix #2 valve

3C. Whip #3: FOR HYDROX

SYSTEM MUST BE PURGED (≤ 2% O_2) BEFORE AND AFTER INTRODUCING HYDROGEN.

FOR ANY MALFUNCTION OR ABORT, FIRST STEP IS TO SECURE HYDROX BOTTLE VALVE. SEE EMERGENCY PROCEDURES.

FLAME PROOF GLOVES MUST BE WORN WHEN HANDLING HYDROX BOTTLE VALVE, CONNECTOR, OR WHIP.

BEWARE!!! HYDROGEN BURNS WITH INVISIBLE FLAME.

NO BOTTLE CONTAINING HYDROGEN SHALL BE VENTED OR OPENED PRECEDING WHIP HOOKUP.

BOTTLE CONTAINING HYDROGEN MUST BE CONNECTED TO WHIP #3 ONLY.

AFTER CONNECTION TO THE WHIP, A GAS BOTTLE CONTAINING HYDROGEN WILL REMAIN CONNECTED UNTIL READY FOR REMOVAL FROM THE LABORATORY.

MONITOR ROOM H_2 LED DISPLAY AT ALL TIMES AFTER CONNECTING HYDROX BOTTLE, MUST BE < 25% LFL.

A GAS BOTTLE CONTAINING HYDROGEN WILL BE DISCONNECTED FROM ITS WHIP USING PROCEDURE 8.

Chamber Control Panel

____ Helium source valve open, pressure indication on helium gauges
____ Inerting line gauge 120 psig, adjust inerting line regulator as necessary
____ Mix source valve closed
____ Shunt valve closed

Mix Gas Bottles and Manifold

_____ Composition/hydrox, must be ≤ 2% O_2
____ Mix #1 and mix #2 valves closed
____ Open mix #3 valve
____ "Bleeding down!" Open inerting valve
____ Open bleed off valve
____ Attach whip #3 to hydrox bottle

Chamber Control Panel

____ Monitor exhaust O_2 LED display until ≤ 2%

Electrical Equipment

____ Switch Teledyne oxygen monitor to LOW range

Chamber Control Panel

____ Monitor exhaust O_2 LED display until ≤ 50 (NOTE: LED is 20 X actual % when Teledyne is in LOW range, e.g. LED 50 ⇒ 2.5% actual O_2)

Mix Gas Bottles and Manifold

____ Close inerting valve
____ Close bleed off valve
____ USING FLAME PROOF GLOVES, SLOWLY open #3 (hydrox) bottle valve, KEEP SAFE DISTANCE!
____ Check whip connector for leaks with Snoop (soap suds) solution
____ Open mix #3 valve
_____ Record manifold pressure, must be 1200 psig minimum
____ USING FLAME PROOF GLOVES, close #3 (hydrox) bottle valve
____ "Bleeding down!" Open bleed off valve
____ Open inerting valve for 1 min, then close
____ Close bleed off valve
____ Close mix #3 valve

Electrical Equipment

____ Switch Teledyne oxygen monitor to HIGH range

4. PROCEDURE: Establish Mix Gas Flow to Saturators

The purpose of this procedure is to provide high O_2 mix gas from bottle #1 to the bubblers.

Electrical Equipment

____ Teledyne oxygen monitor on HIGH

Mix Gas Bottles and Manifold

_____Provide mix gas supply. Open #1 mix gas bottle valve

____ Open mix #1 valve, observe pressure rise on manifold gauge

Chamber Control Panel

____ Shunt valve closed

____ Open mix source valve, observe pressure rise on mix source gauges

____ Adjust mix flow vernier to establish flow rate 2 on flowmeter, monitor differential pressure readout for excessive pressure buildup, visually check saturators for bubbling

____ Load back pressure regulator to 10 psi on differential pressure readout, check for resumption of flow on flowmeter as pressure builds up (adjust mix flow vernier if necessary), visually check saturators for continued bubbling

5. PROCEDURE: Predive Checks

The purpose of this procedures is to confirm that the system is ready prior to high pressure compression.

Supply pressures (psig) must be 0.5 X depth of intended use (fsw) + 200.

Electrical Equipment
____ Hydrogen hood fan on, streamliners are streaming
Right Side Chamber
____ Bubbler select valve positioned as desired
____ Sample valve positioned as desired
____ Ganged sample verniers adjusted for desired flow rate
Synaptosome Perfusion Device
____ Synaptosome filter assembly installed as desired
____ Temperature probe installed in synaptosome heater block, heater block not overheating, verify that temperature controller is cycling
____ Bubbler outlet valves positioned as desired (2 valves)
____ Bleed water trap
____ Beaker at relief valve exhaust is filled with water to a level above the exhaust outlet
____ Close and secure pressure chamber door
Chamber Control Panel
____ Helium source valve open, pressure sufficient to attain desired depth
____ Inerting line gauge 120 psig
____ Mix source valve open, pressure sufficient to attain desired depth
____ Shunt valve closed
____ Saturator differential pressure LED display 10 ± 1 psi, adjust back pressure regulator as necessary
____ Flowmeter mix gas flow rate 2, adjust flow vernier as necessary
____ Adequate fluid level in saturators, good bubbling
____ Room H_2 LED display < 25% LFL
Mix Gas Bottles and Manifold
____ Mix #1 valve open
____ Mix #2 and #3 valves closed

6. PROCEDURE: Dive the Chamber

The purpose of this procedure is to compress the chamber to high pressure.

Predive Notes
Compression valves are opened as necessary for travel according to compression schedule.

Monitor chamber ambient temperature. Synaptosome heater block power supply may be turned off during compression in which case the temperature controller meter will show chamber ambient temperature. Synaptosome heater block power supply may be manually cycled on and off if the ambient temperature during compression is not sufficient to maintain the warmth of the preparation. Power supply should be turned on again at completion of compression.

Ventilate with compression and decompression valves as needed to maintain chamber ambient temperature < 37°C.

Monitor perfusion flow rate and adjust ganged verniers as needed.

Monitor fluid level in saturators and switch on Milton Roy pump as needed to replenish to desired level.

Monitor differential pressure indicator during travel to ensure that saturator pressure does not build up over 10 psi, adjust back pressure regulator as required.

Anticipate increase mix gas flow rate during travel, adjust mix flow vernier for desired flow rate.

For hydrogen dives, check through view port during travel that relief valve exhaust in beaker is not bubbling, i.e., relief valve is seated, prior to switch to hydrox.

Anticipate mix gas switches during travel:
Switch to 2% heliox or trimix at 230 FSW (Procedure 6A).
Switch to hydrox at 340 FSW (Procedure 6B).

Monitor exhaust O_2 LED display at all times when hydrogen is in the system, must be ≤ 50 (Teledyne oxygen monitor in LOW setting; LED display indication is 20 X actual %).

Monitor room H_2 LED display at all times, must be < 25% LFL. If not, activate Emergency Procedure.

Mix gas will be secured immediately on completion of the experiment and prior to decompression by closing the respective bottle valve (Procedure 7). This is especially important for hydrox.

6A. Switch to Mix Gas Bottle #2 ($\leq$ 2% O_2), passing 230 FSW

The purpose of this procedure is to switch to mix gas on whip #2 ($O_2 \leq 2\%$ in inert gas). Provides system purge prior to switch to hydrox.

Chamber Control Panel
____ Close mix source valve
Mix Gas Bottles and Manifold
____ Close #1 bottle valve
____ "Bleeding down!" Open bleed off valve to bleed down, then close
____ Close mix #1 valve
____ Open #2 bottle valve
____ Open mix #2 valve
Chamber Control Panel
____ Open mix source valve

6B. Switch to Mix Gas Bottle #3 (HYDROX), passing 340 FSW

The purpose of this procedure is to introduce mix gas containing hydrogen to the bubbler saturators.

HYDROGEN MUST NOT BE INTRODUCED UNTIL PASSING DEPTH 330 FSW.

SYSTEM MUST BE PURGED WITH MIX GAS CONTAINING ≤2% O_2 PRIOR TO INTRODUCING HYDROGEN.

Left Side Chamber
____ Check relief valve exhaust through view port, no bubbling
Chamber Control Panel
____ Exhaust O_2 LED display ≤ 2%
____ Shunt valve closed
Electrical Equipment
____ Switch Teledyne oxygen monitor to LOW
Chamber Control Panel
____ Exhaust O_2 LED display ≤ 50 (reading is 20 X actual % when Teledyne is in LOW setting)
Mix Gas Bottles and Manifold
____ #1 bottle valve closed
____ Mix #1 valve closed
____ Mix #2 valve open
____ Mix #3 valve closed
____ Close #2 bottle valve
____ Close mix #2 valve
____ USING FLAME PROOF GLOVES, SLOWLY open #3 (hydrox) bottle valve, KEEP SAFE DISTANCE!
____ Open mix #3 valve
Chamber Control Panel
____ Check differential pressure LED display 10 ± 1 psi, adjust back pressure regulator as necessary
____ Check flowmeter mix gas flow rate, adjust flow vernier as necessary
____ Check relief valve exhaust through view port, no bubbling
____ Monitor exhaust O_2 LED display, must be ≤ 50 (Teledyne in LOW setting) until completion of decompression
____ Monitor room H_2 LED display, must be < 25% LFL

7. PROCEDURE: Decompression

The purpose of this procedure is to secure mix gas and to decompress the chamber.

HYDROX IS SECURED AT THE MANIFOLD IMMEDIATELY UPON COMPLETION OF THE EXPERIMENT.

FOLLOWING A HYDROGEN DIVE, MONITOR EXHAUST O_2 LED DISPLAY ($\leq$ 50 when Teledyne in LOW range) AND ROOM H_2 LED DISPLAY (< 25% LFL) UNTIL DECOMPRESSION HAS BEEN COMPLETED AND CHAMBER DOOR IS OPEN.

Mix Gas Bottles and Manifold

____ Close bottle valve in use on completion of the experiment. FLAME PROOF GLOVES must be used if closing #3 (hydrox) bottle valve

Chamber Control Panel

____ "Bleeding down!" Open decompression valves as necessary

____ Unload back pressure regulator, turn slotted screw full CCW

Following 6 steps must be performed following any hydrogen dive

Chamber Control Panel

____ Close mix source valve

____ Open shunt valve, adjust flow vernier as necessary for flow rate 2 on flowmeter

____ Exhaust O_2 LED display < 50 (Teledyne in LOW range)

Mix Gas Bottles and Manifold

____ "Bleeding down!" Open bleed off valve

____ Open inerting valve for 10 s, then close

____ Close mix #3 valve

____ Open mix #2 valve to bleed down, then close

____ Close bleed off valve

On arrival at surface

Chamber Control Panel

____ Close shunt valve if open

____ Close mix source valve if open

____ Close decompression valves

Mix Gas Bottles and Manifold

____ Close mix #1 or mix #2 valve if open

Electrical Equipment

____ Switch Teledyne oxygen monitor to HIGH range

8. PROCEDURE: Shutdown

The purpose of this procedure is to disconnect the hydrox bottle from the system and secure the system

FLAME PROOF GLOVES MUST BE WORN WHEN HANDLING HYDROX BOTTLE VALVE, CONNECTOR, OR WHIP. **BEWARE!!!** HYDROGEN BURNS WITH INVISIBLE FLAME.

Electrical Equipment
____ Turn off heater power supplies 3 (each)
____ Turn off temperature controllers (3 each)
____ Turn off Teledyne oxygen monitor
Chamber Control Panel
____ Mix source valve closed
____ Shunt valve closed
Mix Gas Bottles and Manifold
____ All mix valves closed (3 valves), manifold pressure gauge 0
____ All bottle valves closed
____ USING FLAME PROOF GLOVES, disconnect whip #3 from hydrox bottle, replace plastic insert in valve orifice, replace protective cap over bottle valve, and remove bottle to outdoor storage area
____ Turn off hydrogen hood exhaust fan
____ Drain saturators, use Procedure 2A
____ Refill saturators with distilled water, use Procedure 2B
____ Drain water trap
Helium Supply Wall Panel
____ Close bypass valve
Chamber Control Panel
____ Open bleed off valves (2 valves)
____ "Bleeding down!" Open shunt valve to bleed off helium pressure, then close
____ Close bleed off valves (2 valves)
____ Close helium source valve
____ All pressure gauges 0 (6 gauges)
____ Differential pressure LED display 0 ± 0.2 psi
Helium Supply Wall Panel
____ Delivery pressure 0 (gauge 2)
____ Notify gas farm 5-5910 or duty diver 5-1839 to secure lab helium

APPENDIX B

EMERGENCY PROCEDURES INVOLVING HYDROGEN GAS

Contents

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EMERGENCY PROCEDURES INVOLVING HYDROGEN GAS

GENERAL SAFETY RULES:

FOR ANY MALFUNCTION OR ABORT, FIRST STEP IS TO SECURE HYDROX BOTTLE VALVE, IF POSSIBLE.

FLAME PROOF GLOVES MUST BE WORN WHEN HANDLING HYDROX BOTTLE VALVE, CONNECTOR, OR WHIP.

BEWARE!!! HYDROGEN BURNS WITH INVISIBLE FLAME.

EMERGENCY PROCEDURES ARE PERFORMED BY THE CHAMBER OPERATOR WITH BACKUP BY THE SUPERVISOR.

1. FIRE OR EXPLOSION

If possible, close hydrox bottle valve using flame proof gloves.
If time permits, attempt to isolate hydrogen source by closing
#3 mix valve on manifold panel and/or mix source valve on
chamber control panel.
DO NOT use fire extinguisher (may create explosive condition).
Evacuate lab.
Activate fire alarm system.
Evacuate building.

2. HYDROGEN GAS LEAK

This could be indicated by LFL > 25% on room H_2 LED display, by audible alarm and/or illumination of H or L light on Sensidyne unit, or by operator suspicion.

Close hydrox bottle valve using flame proof gloves.
Close #3 mix valve.
Close mix source valve.
Open inerting valve on chamber control panel.
Check hydrogen hood exhaust fan on, streamers streamlining.
If chamber is pressurized, open shunt valve, open decompression valve, unload back pressure regulator.
When chamber on the surface, open shunt valve (if not already open), bleed off valves on chamber control panel (2 valves), and bleed off valve on manifold panel. Then open #3 mix valve and inerting valve on manifold panel.

3. EXCESS OXYGEN LEVEL DURING HYDROGEN USE

This could be indicated by O_2 > 50 on exhaust O_2 LED display (reading is 20 X actual % when Teledyne oxygen monitor is in LOW setting) or by operator suspicion.

Close hydrox bottle valve using flame proof gloves.
Close #3 mix valve.
Close mix source valve.
Open inerting valve on chamber control panel.
Open shunt valve.
Open decompression valve.
Unload back pressure regulator, slotted screw full CCW.
Monitor exhaust O_2 LED display to maintain $O_2 \leq 50$ (Teledyne in LOW range).
When chamber reaches surface, open bleed off valves on chamber control panel (2 valves) and bleed off valve on manifold panel. Then open #3 mix valve and inerting valve on manifold panel.
Continue system purge to maintain $O_2 \leq 50$ (Teledyne in LOW range), 10 minute minimum.
Secure operations until cause of malfunction is determined and corrected.

4. FAILURE OF GAS TIGHT INTEGRITY OF BUBBLER SYSTEM WITH HYDROGEN LEAK INTO COMPRESSION CHAMBER

This could be indicated by loss or inability to maintain differential pressure in bubblers, by bubbling into beaker at the bubbler relief valve exhaust, or by operator suspicion.

Close hydrox bottle valve using flame proof gloves.
Close #3 mix valve.
Close mix source valve.
Open shunt valve.
Open decompression valve.
Open compression valve to ventillate chamber, monitoring depth to assure continued decompression.
Unload back pressure regulator, slotted screw full CCW.
When chamber reaches surface, open bleed off valves on chamber control panel (2 valves) and bleed off valve on manifold panel. Then open #3 mix valve and inerting valve on manifold panel.
Continue ventillation of chamber for 30 min after reaching surface prior to opening chamber door.
Secure operations until cause of malfunction is determined and corrected.

5. ABORT DIVE

Close hydrox bottle valve using flame proof gloves.
Close #3 mix valve.
Close mix source valve.
Open shunt valve.
Open decompression valve.
Unload back pressure regulator, slotted screw full CCW.
When chamber reaches surface, open bleed off valves on chamber control panel (2 valves) and bleed off valve on manifold panel. Then open #3 mix valve and inerting valve on manifold panel.
Secure operations until cause of malfunction is determined and corrected.

6. UNCONTROLLED CHAMBER PRESURE INCREASE

Close hydrox bottle valve using flame proof gloves.
Close compression stop valve left side of chamber.
Close mix in stop valve left side of chamber.
Close helium source valve chamber control panel.
Close mix source valve chamber control panel.
Close all mix valves on manifold panel.
Close all valves on helium supply wall panel.

APPENDIX C

CHECKLIST FOR NON-HYDROGEN DIVES

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Synaptosome Chamber Dive Checklist (Rev Mar 91)

NOT TO BE USED FOR PROCEDURES INVOLVING HYDROGEN GAS

Date: _____
Person in charge: _____
Persons assisting, if any: _____

Check off each item as performed.
Mark NR if step is determined not required by person in charge
Note any discrepancies

1. Preliminary Checks

Helium Supply Wall Panel

____ All valves closed (4 valves)
____ Regulator unloaded (blue knob CCW)
____ Upstream pressure 1,200 +/- 100 psi (gauge 1)
____ Delivery pressure 0 (gauge 2)

Saturation Mix Gas Bottles and Manifold

____ All gas bottles secured
____ All gas bottle valves off
____ All manifold valves off
____ Manifold pressure gauge 0

Chamber Control Panel

____ Helium source valve closed
____ Mix source valve closed
____ All pressure gauges 0 (6 gauges)

Synaptosome Perfusion Device

____ Electrical connectors installed (3 connectors interface to chamber)
____ Temperature sensors and heater elements plugged in (3 of each)
____ Quick disconnect fittings installed (red = mix in, 3 greens = mix out from top of water trap, mix out from each saturator, 2 violets = buffer 1 to R saturator, buffer 2 to L saturator)
____ Temperature sensor installed in synaptosome heat block

2. Valve Lineup

Helium Supply Wall Panel

____ Recheck 4 valves closed and delivery supply pressure 0

Saturation Mix Gas Bottles and Manifold

____ Recheck gas bottle valves closed, manifold valves closed, and manifold pressure gauge 0

Milton Roy Pump

____ Pump off
____ Pump vernier controls 0 (2 knobs, full CCW)
____ Pump flush valves closed (2 valves)
____ Pump select valves to drain (2 valves)

Chamber Control Panel

____ Recheck all pressure gauges 0 (6 gauges)
____ Differential pressure readout on, indicating 0 +/- 0.2 psi

_____ Gauge calibration port capped and valve closed
_____ Gauge bleed off valve closed (black)
_____ Helium source valve closed (black)
_____ Mix source valve closed (red)
_____ Shunt valve closed (red)
_____ Bleed off valves closed (red, 2 valves)
_____ Mix flow vernier closed
_____ Back pressure regulator unloaded (set screw full CCW)
_____ Compression valves closed (2 valves, X = coarse, black = fine)
_____ Decompression valves closed (2 valves, X = coarse, black = fine)
_____ Inerting valve closed (black)
_____ Flow meter valve full open, turns freely (black)

Top Chamber

_____ Fast decompression line closed (2 quarter turn valves, both closed)

Left Side Chamber

_____ Analysis valve closed
_____ Depth gauge valve open
_____ Reference valve open
_____ Decompression valve open
_____ Compression valve open
_____ Mix in valve open
_____ Mix out valve open
_____ Buffer 1 valve open
_____ Buffer 2 valve open

Synaptosome Perfusion Device

_____ perfusion valves closed (2 valves, tagged yellow)

Right Side Chamber

_____ Sample valve off
_____ Ganged sample verniers open, turn freely (2 valves)
_____ Bubbler select valve off

3. Fill Saturators

Electrical Equipment

_____ Turn on heater power supplies (3 each)
_____ Turn on temperature controllers (3 each) and check setting 37°

Helium Supply Wall Panel

_____ Provide helium supply. Open bypass valve, note pressure rise on delivery supply gauge

Chamber Control Panel

_____ Open helium source valve, note pressure rise on helium pressure gauges (2 gauges, upper gauge shows supply pressure; lower gauge reflects loading of helium tracking regulator)
_____ Open shunt valve, note pressure rise on mix pressure gauges (2 gauges, upper and lower gauge indications as above)
_____ Establish gas flow through saturators. Open mix flow vernier for flow rate 2 on flowmeter, monitor differential pressure readout for excessive pressure buildup, visually check saturators for bubbling
_____ Establish pressure in saturators. Load back pressure regulator to 25 psi on differential pressure readout, check for resumption of flow in flowmeter as pressure builds up (adjust mix flow vernier if necessary), visually check saturators for

continued bubbling

Milton Roy Pump

____ Drain the saturators. Open pump flush valves (2 valves), visually check for effluent in drain lines

____ When saturators are drained, close pump flush valves (2 valves)

____ Prime Milton Roy pump. Turn on pump and prime with solutions of choice, adjusting pump vernier controls as required (2 knobs); check pump select valves in drain position (2 valves) and effluent flowing in drain lines

____ Refill saturators. After pump is primed, switch pump select valves to bubbler position

____ Visually monitor saturators to prevent overfilling

____ When saturators are filled, turn off Milton Roy pump and return pump vernier controls to 0 (2 knobs)

Electrical Equipment

____ Ensure that temperature controllers are cycling and that heating elements on perfusion device are not overheating

Chamber Control Panel

____ Secure gas flow through saturators. Unload back pressure regulator, turn slotted screw full CCW, observe pressure drop on differential pressure indicator

____ Close mix flow vernier

____ Close helium source valve

____ Vent control panel lines. Open bleed off valves (2 valves), note pressure drop on all helium and mix pressure gauges (4 gauges), close both bleed off valves when venting is complete

____ Close shunt valve

4. Establish Mix Gas Flow to Saturators

____ Check and record tank pressures of each mix gas to be used on the dive

Saturation Mix Gas Bottles and Manifold

Tank #1	Tank #2	Tank #3	
_____	_____	_____	Composition (e.g. 21% HeO2)
_____	_____	_____	Open Tank valve
_____	_____	_____	Open Manifold valve
_____	_____	_____	Record manifold pressure, must be sufficient to provide gas flow at the depth pressure at which mix will be used
_____	_____	_____	Close manifold valve (tank valve stays open)

Chamber Control Panel

_____	_____	_____	Open mix supply valve
_____	_____	_____	Open upper bleed off valve to vent the line, and close when vent complete
_____	_____	_____	Close mix supply valve

Saturation Mix Gas Bottles and Manifold

____ Provide mix gas supply. Open manifold valve for selected mix gas, observe pressure rise on manifold gauge

Chamber Control Panel

____ Open mix source valve, observe pressure rise on mix source gauges

____ Open mix flow vernier to establish flow rate 2 on flowmeter,

monitor differential pressure readout for excessive pressure buildup, visually check saturators for bubbling

____ Load back pressure regulator to 10 psi on differential pressure readout, check for resumption of flow on flowmeter as pressure builds up (adjust mix flow vernier if necessary), visually check saturators for continued bubbling

5. Predive Checks

____ Bubbler select valve positioned as desired
____ Synaptosome filter assembly installed as desired
____ Temperature probe installed in synaptosome heating block, heating block not overheating, reverify that temperature controller is cycling
____ Saturator perfusion outlet valves positioned as desired (2 valves)
____ Sample valve positioned as desired
____ Ganged sample verniers adjusted for desired flow rate
____ Close and secure pressure chamber door

6. Dive the Chamber

Predive Notes

Open and adjust compression valves as necessary for travel
Record synaptosome temperature every minute
Ventillate with decompression valves as needed to maintain temperature < 39.5°
Monitor differential pressure indicator during travel to ensure that saturator negative pressure does not build up over 25 psi
Monitor perfusion flow rate and adjust ganged verniers as needed to keep rate constant
Anticipate mix gas switch during travel
Anticipate increase mix gas flow rate during travel
Record above two items respectively in 6a and 6b and flag in travel schedule

6a. Mix Gas Switch

_____ Depth passing for mix gas switch

Saturation Mix Gas Bottles and Manifold

____ Close manifold valve of mix gas tank presently in use
____ Observe pressure drop in manifold gauge
____ When manifold pressure drops below recorded pressure of mix gas to be used next, open its manifold valve

6b. Mix Gas Flow Rate Increase

_____ Depth passing for mix gas flow rate increase

Chamber Control Panel

____ Open flow rate vernier to increase flow rate to 10 on accessory flowmeter scale

6. Commence Dive

Chamber Control Panel

____ Open helium supply valve, note pressure rise on gauges (2 gauges)
____ COMPRESSION

APPENDIX D

SYSTEM PHOTOGRAPHS: CONTROLS NOMENCLATURE AND ACTIONS

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Helium Supply Wall Panel

Provides high pressure He service to the laboratory.

A1. Bypass valve. Bypasses the regulator to provide unregulated high pressure He service.

A2. Gauge 2, delivery pressure gauge. Indicates the pressure of the He service provided.

A3. Gauge 1, upstream pressure gauge. Indicates the source pressure of He supply that is piped to the laboratory.

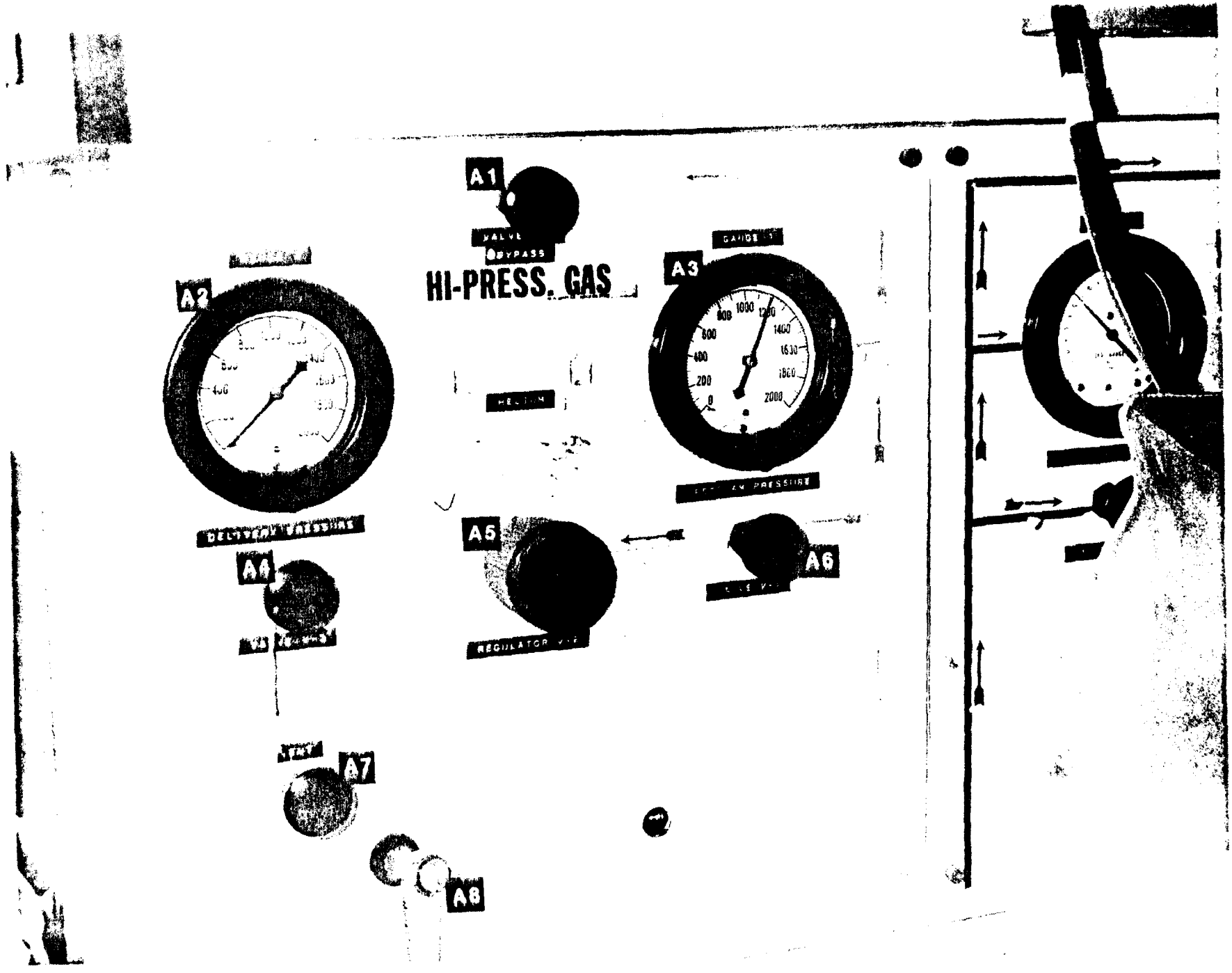
A4. Regulated gas supply valve. Introduces He from the regulator for laboratory service.

A5. Regulator. Controls pressure from the He supply source to provide adjustable pressure for laboratory service.

A6. Supply valve. Introduces high pressure He from the source that is piped to the laboratory to the regulator.

A7. Vent valve. Permits bleeding down laboratory service lines to relieve pressure following use.

A8. Capped connector. Auxiliary connector for He service.


A1
VALVE
BYPASS
HI-PRESS. GAS
A2
A3
A4
A5
A6
A7
A8

Milton Roy Pump

A ganged dual high pressure liquid pump to replenish solutions contained in the saturator vessels of the perfusion apparatus inside the compression chamber. Associated circuitry permits draining the saturators. Drainage is driven by pressurization of the saturators.

B1. Bubbler 2 drain valve. Opens to drain bubbler 2.

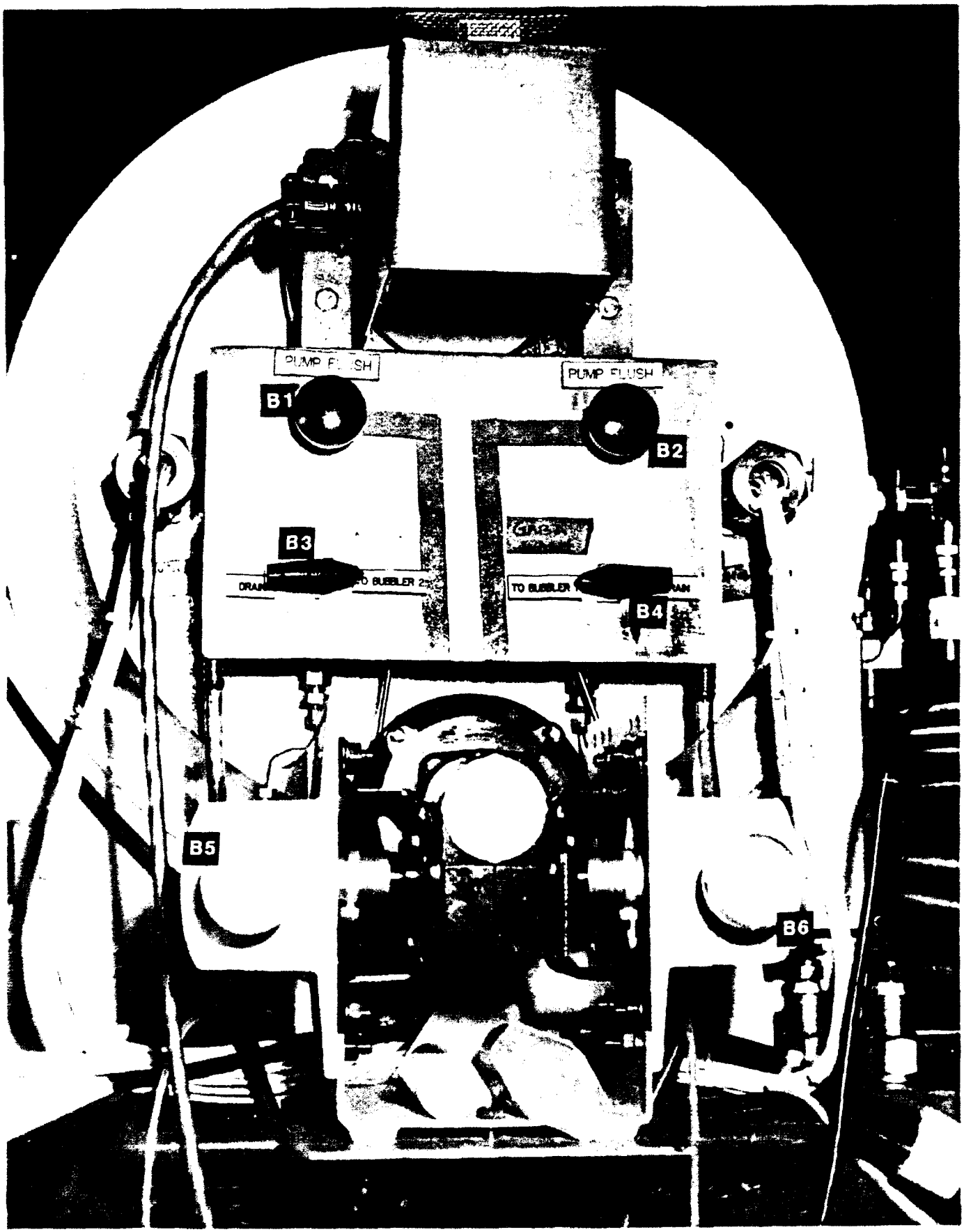
B2. Bubbler 1 drain valve. Opens to drain bubbler 1.

B3. Pump 2 selector valve. Introduces output of pump 2 to bubbler 2 or alternatively shunts pump 2 to drain. Shunt allows lines to be primed with a physiologic solution or to dump when only the ganged mating pump is being used.

B4. Pump 1 selector valve. See item B3.

B5. Pump 2 stroke control. Selects stroke volume of pump 2 from 0-100%.

B6. Pump 1 stroke control. See item B5.


PUMP FLUSH
PUMP FLUSH
B1
B2
B3
B4
B5
B6

Mix Gas Bottles and Manifold: Bottles

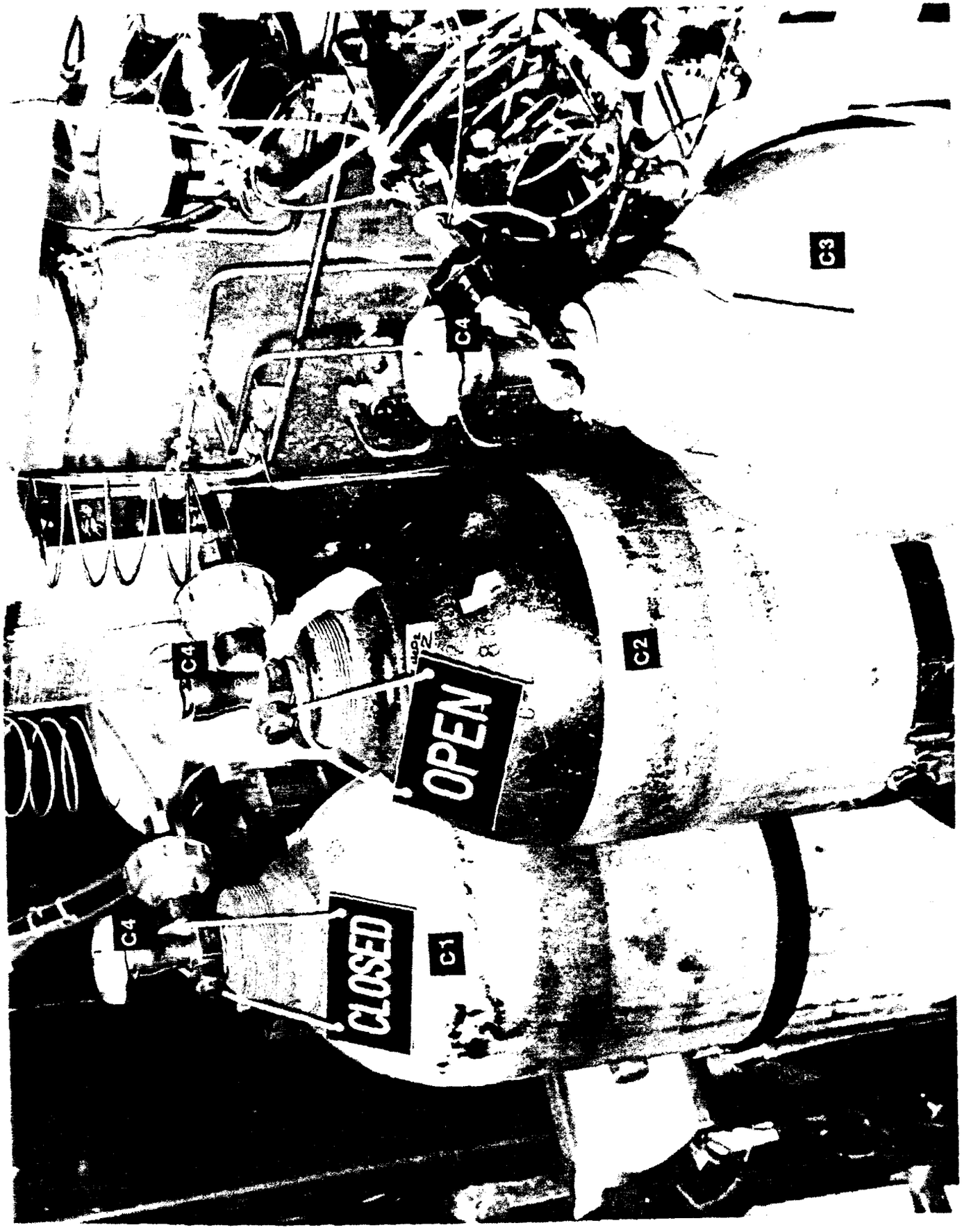
Source of gas mixtures used to bubble saturate the perfusion media that bathes the tissue preparation.

C1. Gas bottle #1, connected to whip #1. This position is used for mixtures not containing H_2, typically with O_2 fractions up to 25%.

C2. Gas bottle #2, connected to whip #2. This position is reserved for mixtures not containing H_2 and with O_2 fractions 2% or less. This mixture is used as an intermediate flush prior to introducing H_2 mixtures to the system.

C3. Gas bottle #3, connected to whip #3. The position is reserved exclusively for mixtures containing H_2.

C4. Gas bottle valves.


C3
C4
C4
C2
OPEN
C4
C1
CLOSED

Mix Gas Bottles and Manifold: Manifold

Common distribution point for any of three different gas mixtures that can be selected to bubble the perfusion media. Circuitry contains no-backflow check valves to prevent contamination of the various gas sources from the other sources.

D1. Mix 1 manifold valve (green color). Introduces gas from whip #1 to the system. See item C1.

D2. Mix 2 manifold valve (green color). Introduces gas from whip #2 to the system. See item C2.

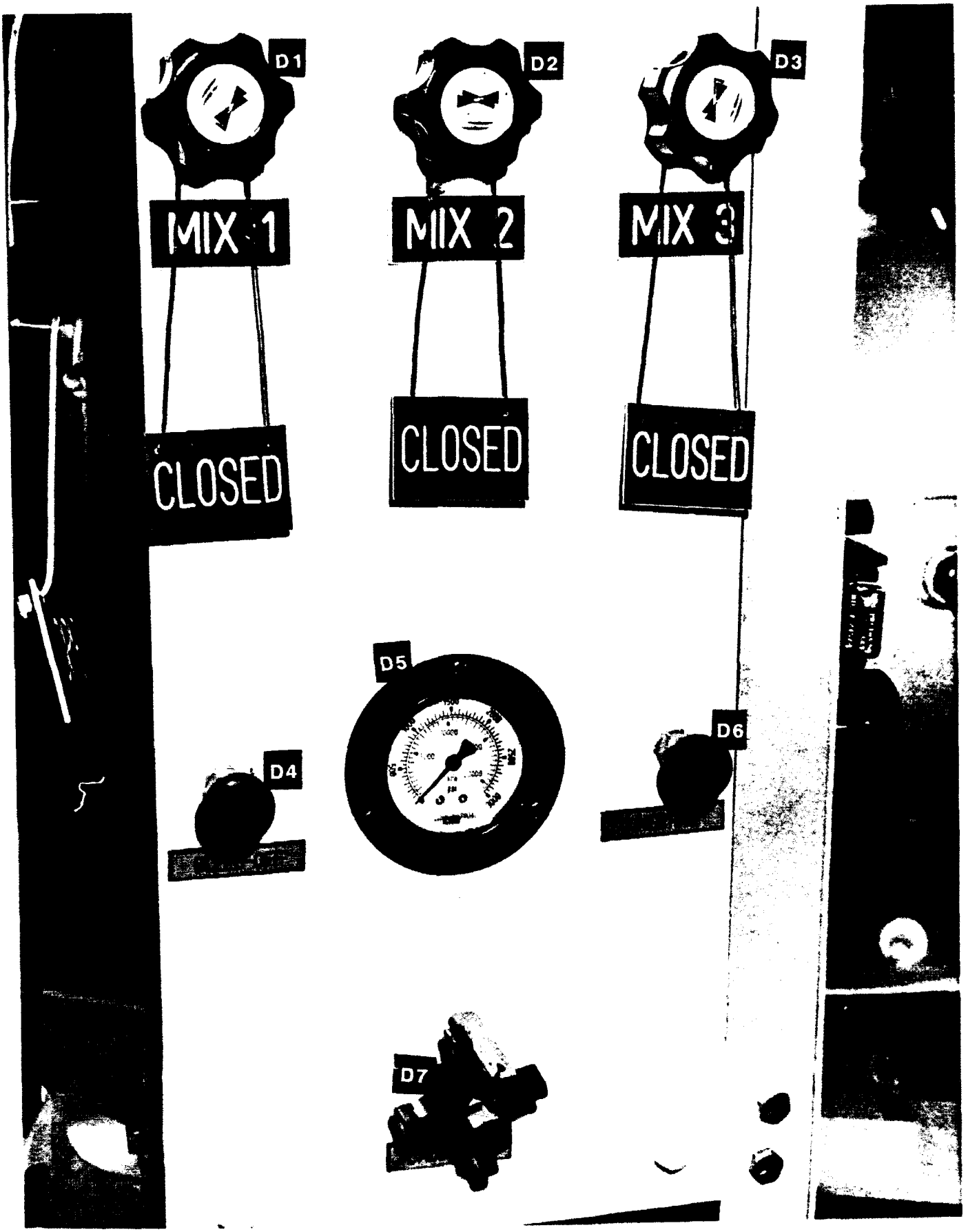
D3. Mix 3 manifold valve, for H_2 mixtures (red color). Introduces gas from whip #3 to the system. See item C3.

D4. Bleed off valve. Permits bleeding down manifold to relieve pressure following use. The inerting valve may be concurrently opened to purge H_2 if needed. See item D6.

D5. Manifold pressure gauge. Displays pressure in the gas mixture manifold.

D6. Inerting valve. Introduces He from service supply to whip #3. Permits purging the H_2 whip during connection/disconnection.

D7. Gauge calibration port and valve. Capped port for access to calibrate the manifold pressure gauge.


D1
MIX 1
CLOSED
D2
MIX 2
CLOSED
D3
MIX 3
CLOSED
D4
D5
D6
D7

Chamber Control Panel

System controls for diving the compression chamber and adjusting pressures and flows in the bubble saturators

E1. Master depth gauge. Indicates depth of the chamber in fsw.

E2. Auxiliary depth gauge. LED readout of chamber depth is fsw.

E3. Gauge bleed off valve. Permits bleeding down master depth gauge lines to relieve pressure following calibration or at other times when gauge is isolated from system

E4. Ball flowmeter. Indicates gas flow rate through saturator vessels.

E5. Flowmeter valve. Introduces gas from the exhaust of the saturator vessels to the flowmeter.

E6. Exhaust O_2 LED display. Slaved to Teledyne O_2 monitor in the electrical equipment rack. Displays % scale reading of the Teledyne meter. Directly reads $\%O_2$ in the exhaust stream when Teledyne is in the LOW setting. When Teledyne is in the HIGH setting, LED 50 → 2.5% O_2 in the exhaust stream. The exhaust contains gas mixture from the saturator vessels, decompression gas from the chamber, and any added inerting gas.

E7. Room H_2 LED display. Slaved to Sensidyne H_2 monitor in the electrical equipment rack. Displays %LFL (lower flammability limit) H_2 sensed in room air passing into the forced air ventilation hood above the chamber control panel.

E8. Chamber temperature LED display.

E9. Gauge calibration port and valve. Capped port for access to calibrate the master depth gauge.

E10. Helium source valve (black color). Introduces He from the laboratory service supply to the system for chamber compression and inerting usage.

E11. Helium delivery supply gauge. Indicates pressure of laboratory service He provided to the system.

E12. Helium tracking regulator adjustment access. He for chamber compression is supplied through a tracking regulator which is referenced to the ambient chamber pressure. The regulated supply pressure relative to chamber pressure is set by hex screw adjustment of the tracking regulator through this access hole.

E13. Helium compression tracking-regulated supply gauge. Indicates loading of the tracking regulator to supply He for compressing at pressure that is referenced to current chamber pressure. See item E12.

E14. Compression valve, coarse (X handle). Compresses the chamber.

E15. Compression valve, fine (black color). Fine adjustment to chamber compression downward.

E16. Shunt valve (red color). Connects laboratory service He supply to saturator vessel system. Used to purge the system with He. May also be used for convenience to pressurize the saturator vessels, e.g., to drain liquids. Circuitry contains no-backflow check valves to prevent contamination of He service supply by mixed gas.

E17. Mix source valve (red color). Introduces gas from the mix gas manifold to the saturator vessel system.

E18. Mix source supply gauge. Indicates pressure supplied from the mix gas manifold to the saturator vessel system.

E19. Mix gas tracking regulator adjustment access. Gas mixture for the saturator vessel system is supplied through a tracking regulator which is referenced to the ambient chamber pressure. The regulated supply pressure for mix gas relative to chamber pressure is set by hex screw adjustment of the tracking regulator through this access hole.

E20. Mix gas tracking-regulated supply gauge. Indicates loading of the tracking regulator to supply gas mixture for the saturator vessel system at pressure that is referenced to current chamber pressure. See item E19.

E21. Bleed off valves (2 valves, red color). Permits bleeding down system lines to relieve pressure following use.

E22. Back pressure regulator. Regulates pressure in the exhaust stream from the saturator vessels referenced to the ambient pressure of the chamber so that a differential pressure is maintained in the saturators above the chamber pressure. Adjustment of the differential pressure in the saturator vessels is set by the slotted screw.

E23. Differential pressure LED display. Indicates the differential pressure in the saturator vessels above chamber pressure. Indication is in psi. Differential pressure is limited to 10 psi when using H_2 to reduce chances for leaking from the saturators into the chamber. Inability to set or maintain differential pressure suggests leak of the closed saturator vessel system.

E24. Mix flow vernier. A fine metering valve which adjusts flow rate of mix gas through the saturator system, as indicated at the ball flowmeter. See item E4.

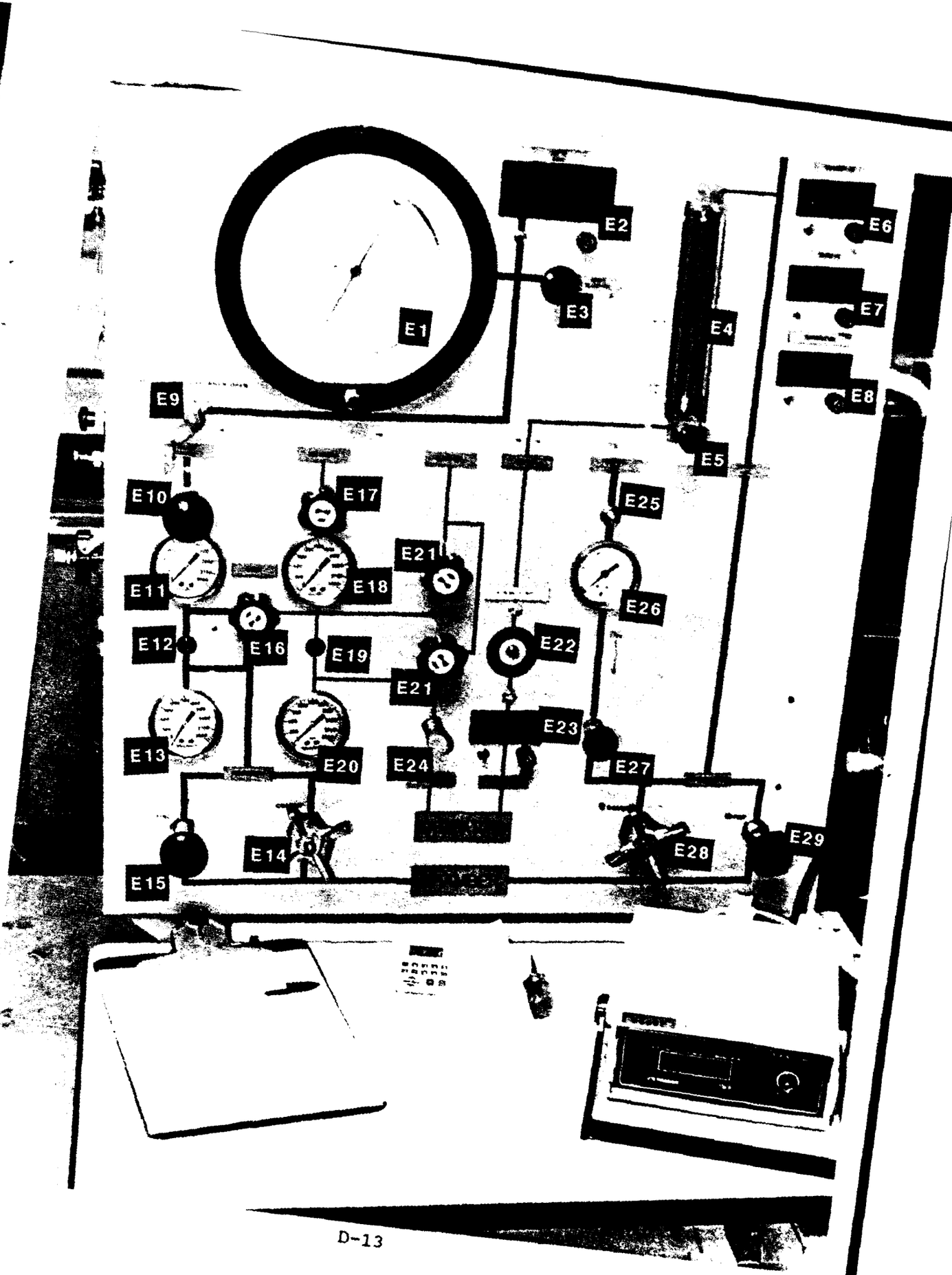
E25. Inerting line regulator. He for exhaust dilution to inert the exhaust stream when using H_2 is supplied from the He service supply. Regulation for the He to dilute the exhaust stream is set by the slotted screw adjustment of this regulator.

E26. Inerting line gauge. Shows regulated He pressure for diluting the exhaust stream. Set to 120 psi. See item E26.

E27. Inerting valve (black color). Opens the inerting line to dilute O_2 in the exhaust stream from the saturator vessel system and the chamber decompression. Used during H_2 operations. See item E7.

E28. Decompression valve, coarse (X handle). Decompresses the chamber.

E29. Decompression valve, fine (black color). Fine adjustment to chamber decompression upward.


E1
E2
E3
E4
E5
E6
E7
E8
E9
E10
E11
E12
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E18
E19
E20
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E24
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E26
E27
E28
E29

Top Chamber

F1. Fast decompression line. Stop valve shown. Another valve is also located on the line.

Left Side Chamber

F2. Analysis valve. Stop valve, not connected.

F3. Depth gauge valve. Stop valve to depth gauge line.

F4. Reference valve. Stop valve to reference lines to tracking regulators and back pressure regulator.

F5. Decompression valve. Stop valve to decompression exhaust line.

F6. Compression valve. Stop valve to He compression supply.

F7. Mix in valve. Stop valve to mix gas supply for saturator vessels.

F8. Mix out valve. Stop valve to exhaust from saturator vessels. Gas tight integrity of saturator vessels is indicated when vessels maintain differential pressure above the ambient chamber pressure with mix in and mix out valves closed.

F9. Buffer 2 valve. Stop valve for line from Milton Roy pump 2.

F10. Buffer 1 valve. Stop valve for line from Milton Roy pump 1.

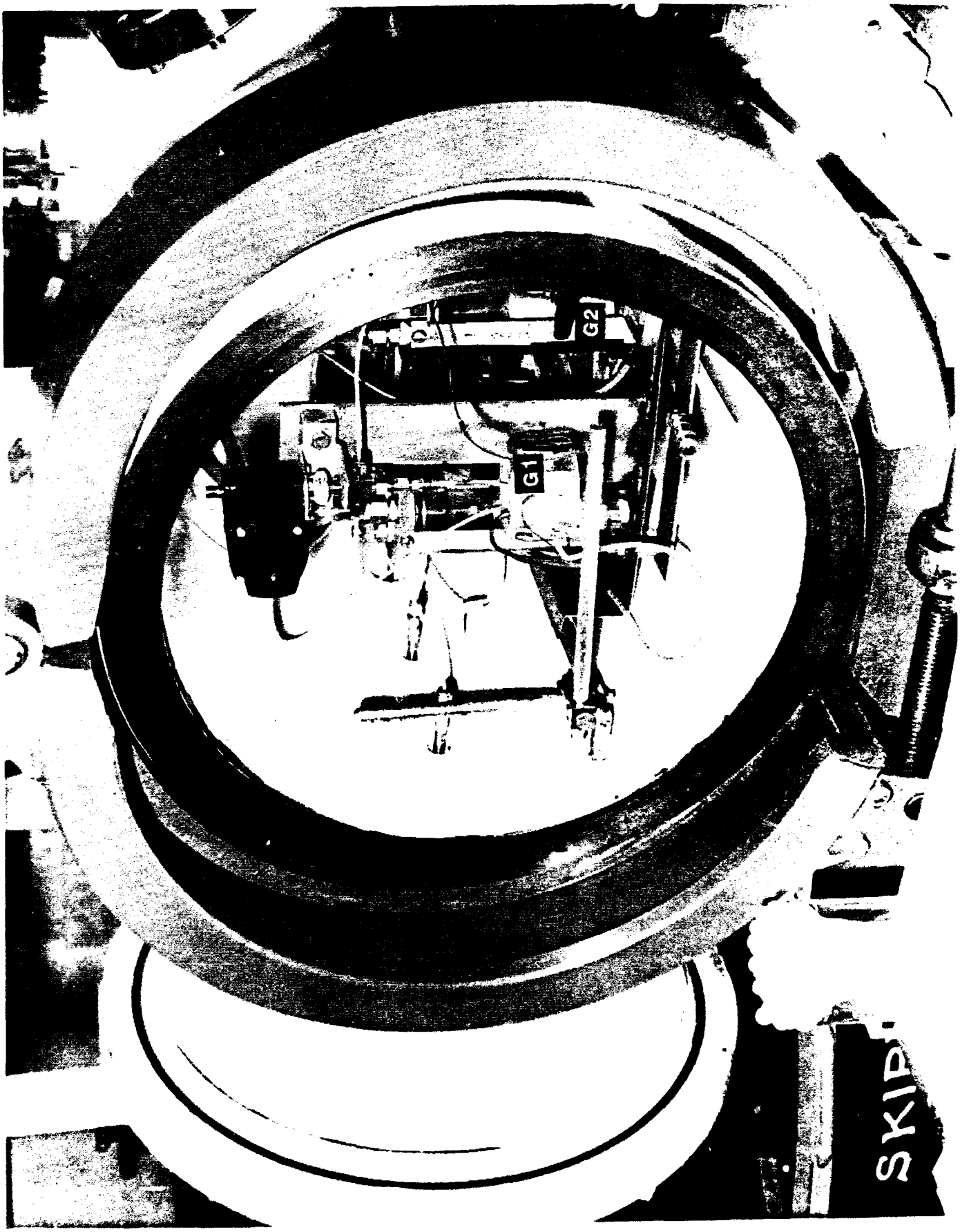
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10

Synaptosome Perfusion Device

The device is located inside the chamber.

G1. Synaptosome heater block. Thermostated to maintain temperature of the tissue preparation.

G2. Bubbler outlet valve. Shuts off liquid flow from saturator vessel so that the tissue preparation may be serviced.


G2
G1
SKIP

Right Side Chamber

H1. Sample valve. Shuts off flow of liquid from perfusion device.

H2. Ganged sample verniers. A dual fine metering valve to control flow rate of liquid from the perfusion device.

H3. Bubbler select valve. Controls a pneumatic valve on the perfusion device to select perfusion of the tissue preparation either from saturator vessel #1 or #2. Switching from low K^+ solution in vessel #1 to high K^+ solution in vessel #2 depolarizes the membranes of the nerve terminal preparation and stimulates neurotransmitter secretion.

H3
H1
H2

Electrical Equipment

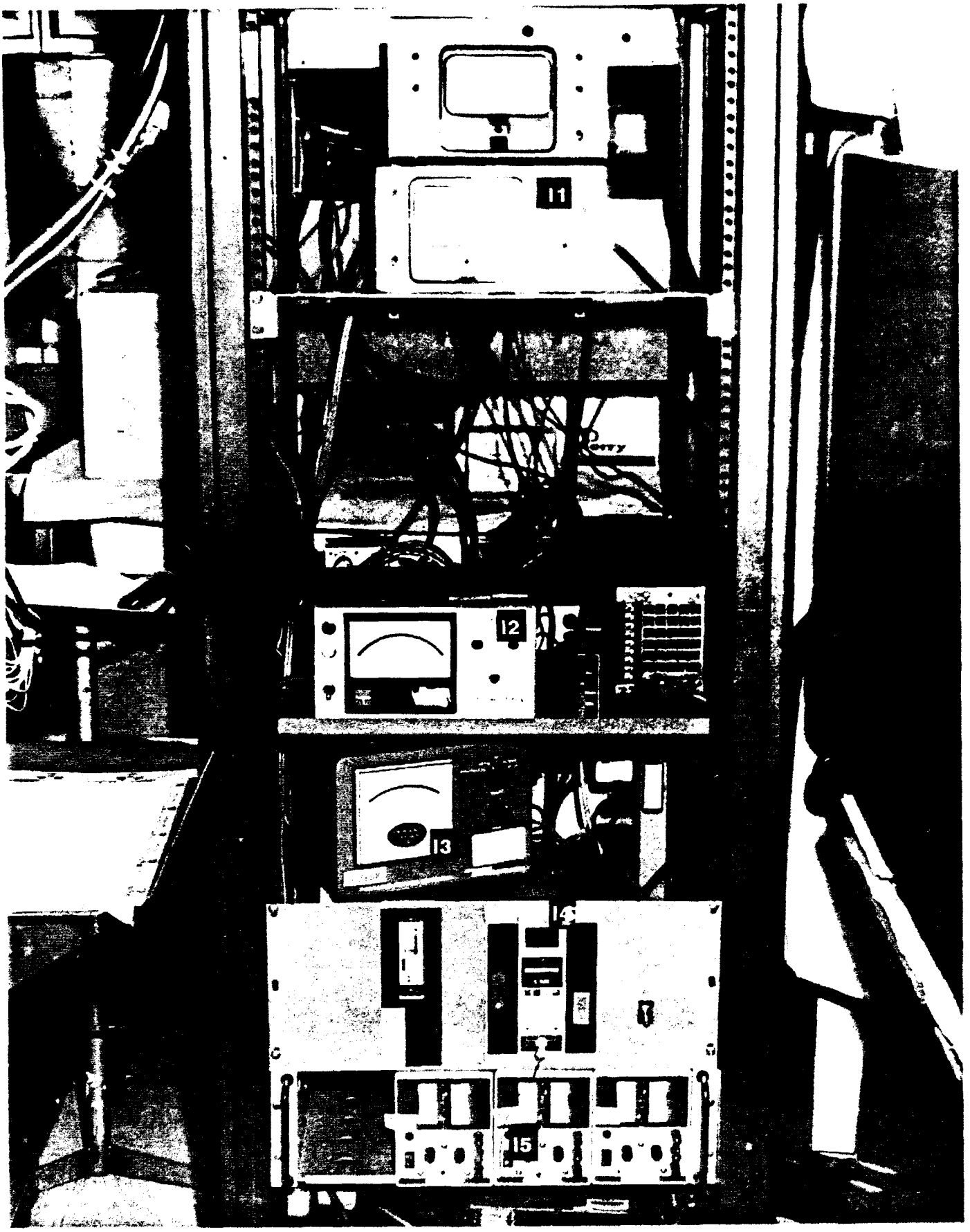
I1. Temperature controllers for saturator vessels. Monitor temperature of liquid in the bubblers and switches on power source for heating when low temp threshold is sensed.

I2. Temperature controller for synaptosome heater block. See Items G1 and I1.

I3. Teledyne O_2 sensor. Measures O_2 in the exhaust from the chamber and system. See item E7. Exhaust gas can not be ignited when O_2 is less that 2%.

I4. Sensidyne H_2 sensor. Measures H_2 in room air passing into the forced air ventilation hood above the chamber control panel. Indication is %LFL (lower flammability limit; LFL is 4% H_2). See item E6.

I5. Heater power supplies. Provide current for controlling temperature of the saturator vessels and the synaptosome heater block.


I1
I2
I3
I5

APPENDIX E

COMPRESSION SCHEDULES

Contents

	Page
Linear Compression to 64 ATA	E-2
Exponential Compression to 2000 fsw	E-3
Exponential Compression to 64 ATA, for H_2	E-4

filename lin_cmpr.wq1

Linear compression profile to 64 ATA

time	fsw	time	fsw	time	fsw
		10	866	20	1733
15 sec	22		888		1754
30 sec	43		910		1776
45 sec	65		931		1797
1	87	11	953	21	1819
	108		975		1841
	130		996		1862
	152		1018		1884
2	173	12	1040	22	1906
	195		1061		1927
	217		1083		1949
	238		1104		1971
3	260	13	1126	23	1992
	282		1148		2014
	303		1169		2036
	325		1191		2057
4	347	14	1213	24	2079
	368		1234		
	390		1256		
	411		1278		
5	433	15	1299		
	455		1321		
	476		1343		
	498		1364		
6	520	16	1386		
	541		1408		
	563		1429		
	585		1451		
7	606	17	1473		
	628		1494		
	650		1516		
	671		1538		
8	693	18	1559		
	715		1581		
	736		1603		
	758		1624		
9	780	19	1646		
	801		1668		
	823		1689		
	845		1711		

Compression profile for synaptosomes to 2000 fsw

time	fsw		time	fsw	time	fsw
			10	154	20	1023
15 sec	1			162		1070
30 sec	3			171		1119
45 sec	5			180		1170
1	6		11	189	21	1223
	8			199		1278
	10			209		1336
	12			220		1397
2	14		12	231	22	1460
	16			243		1527
	18			255		1596
	20			268		1668
3	22		13	281	23	1743
	25			295		1822
	28			309		1904
	30			325		1989
4	33		14	340	24	2000
	36			357		
	39			374		
	42			392		
5	45		15	411		
	49			431		
	53			451		
	56			473		
6	60	:SWITCH	16	495		
	64	GASSES		518		
	69			543		
	73			568		
7	78		17	595		
	83			623		
	88			652		
	93			682		
8	99		18	714		
	105			747		
	111			781		
	117			817		
9	124		19	855		
	131			894		
	138			935		
	146			978		

Compression profile for synaptosomes to 64 ATA (2079 FSW)
(hydrox and trimix study)

time	fsw	time	fsw	time	fsw
		10	154 air 157	20	1023
15 sec	1 21% O2		162		1070
30 sec	3		171		1119
45 sec	5		180		1170
1	6	11	189	21	1223
	8		199		1278
	10		209		1336
	12		220		1397
2	14	12	231 2% HeO2	22	1460
	16		243		1527
	18		255		1596
	20		268		1668
3	22	13	281	23	1743
	25		295		1822
	28		309		1904
	30		325		1989
4	33	14	340 N2 or H2	24	2079
	36		357		
	39		374		Xperiment
	42		392		
					Mix off!
5	45	15	411		
	49		431		Dcompress
	53		451		
	56		473		
6	60	16	495		
	64		518		
	69		543		
	73		568		
7	78	17	595		
	83		623		
	88		652		
	93		682		
8	99	18	714		
	105		747		
	111		781		
	117		817		
9	124	19	855		
	131		894		
	138		935		
	146		978		